

# BIOLOGY AND BEHAVIOUR OF *Elaeidobius kamerunicus* FAUST, 1898 (COLEOPTERA: CURCULIONIDAE) IN TWO OIL PALM CULTIVARS IN COLOMBIA

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## ABSTRACT

*Elaeidobius kamerunicus* is the most efficient and abundant pollinator in oil palm plantations. We recorded biweekly observations to understand the population behaviour, habits and biological features of *E. kamerunicus*, as well as the volatile organic compound profile emitted by the inflorescences of two oil palm cultivars. Samples of the male and female inflorescences during the anthesis of *Elaeis guineensis* and the hybrid *Coari* × *La Mé* were collected. *Elaeidobius kamerunicus* adults copulated between 9:30 and 11:30 hr, and the females oviposited on the stamens of the male flowers. The life cycle of *E. kamerunicus* from egg to pupa took 8.6 days, and the life expectancy of adult males and females was 40.8 and 51.4 days, respectively. The female oviposition rate was 1.3 eggs/day, with a development time of 0.5 days. Insects such as *Pachycondyla harpax*, *Hololepta* sp., and members of the *Chrysopidae* and *Dermaptera* families, as well as spiders and *Pseudoscorpiones*, predated on this species. The main compounds emitted by the male and female inflorescences were methyl salicylate, estragole, *cis*-Anethole, and *trans*-Anethole studied in these two cultivars.

**Keywords:** *Elaeis guineensis*, estragole, life cycle, pollinator, predators.

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## INTRODUCTION

Oil palm is one of the most important crops in Colombia. Its high productivity and perennial nature have driven the oil palm's rapid expansion as a plantation crop. Colombia is the fourth-largest producer of crude palm oil globally, with a contribution of 1 559 065 t/yr and 595 722 ha of land planted with *Elaeis guineensis* Jacq.

(Arecaceae) and the interspecific hybrid (*Elaeis oleifera* × *Elaeis guineensis*) (Fedepalma, 2022). Oil palm is a monoecious plant that requires cross-pollination (Syed, 1984). It is commonly wind-pollinated (anemophilous) or insect-pollinated (entomophilous), with the latter mechanism being the most important.

The most frequent oil palm pollinators worldwide are *Mystrops costaricensis* Gillogly, 1972 (Coleoptera, Nitidulidae), *Elaeidobius subvittatus* (Faust, 1898) (Coleoptera, Curculionidae) and *E. kamerunicus* Faust, 1898 (Coleoptera, Curculionidae) (Mondragón and Roa, 1985; Syed, 1986). Prior to 1985, *M. costaricensis*, native to America, and *E. subvittatus*, native to Africa, were the main pollinating insect species of oil palms that were identified in Colombia (Mondragón and Roa, 1985). However, given the low pollination efficiency of *M. costaricensis* and *E. subvittatus*, it was deemed necessary to introduce the *E. kamerunicus*

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species from Malaysia. According to Syed (1986), *E. kamerunicus* is the most effective pollinator of oil palm crops and has contributed to the increase of oil palm production from 20% to 30% in Malaysia.

Dilleephan (1992) described *E. kamerunicus* male's morphological characters, which are larger than the females. Males have many setae on the outer part of the body, a characteristic absent in females, which helps to increase their body's pollen-carrying capacity (Syed, 1984; Genty, 1985). Unfortunately, improper application of chemical insecticides has caused a reduction of 10% to 20% in the *E. kamerunicus* field populations. *Elaeidobius kamerunicus* is also vulnerable to predation by ants, spiders, termites, and nematodes (Prasetyo *et al.*, 2014) and is sensitive to precipitation patterns. These factors have collectively led to poor cluster formation and low oil palm extraction (Sánchez *et al.*, 2004).

Entomophilic oil palm pollination depends on the number of male inflorescences during anthesis, population size and behaviour of pollinating insects, and pollen availability with good viability and germinability. Efficient pollination of *E. guineensis* inflorescences is represented by a higher production rate of normal or fertile fruit, thereby increasing oil production by decreasing parthenocarpic fruit and abortive flowers (Corley and Tinker, 2003). While in hybrid materials, floral asynchrony and insufficient pollination cause poor conformation of the bunch, loss of bunches, and a higher percentage of parthenocarpic fruits (Hormaza *et al.*, 2011). The emission of volatile compounds that inflorescences emit to attract pollinating insects determines the pollination efficiency. Female inflorescences emit fragrances similar to those emitted by male inflorescences to ensure pollination during anthesis, attracting pollinators carrying pollen grains on their bodies (Lajis *et al.*, 1985; Yue *et al.*, 2015).

Therefore, to achieve the best conformation of fruits and the most significant potential for oil production of two of the most widely planted oil palm cultivars in Colombia, it is necessary to

understand the population behaviour, habits and biological features of the pollinator *E. kamerunicus*, as well as the profile of volatile organic compounds emitted by the inflorescences in order to develop studies with attractant pheromones that improve the formation and increase the number of normal fruits in interspecific hybrids.

## MATERIALS AND METHODS

### Study Area

The study was carried out in the Palmar de la Vizcaína of Cenipalma Experimental Field (CEPV, Campo Experimental Palmar de la Vizcaína de Cenipalma), located 31 km from the Municipality of Barrancabermeja, in the Central Palm Zone of Colombia (06° 59' 1.15" N, 73° 41' 43.35" W, 100 m asl). On the plantation, two oil palm plots, each measuring 2 ha, were selected. One of the plots consisted of *E. guineensis* cultivar (IRHO 1001) planted in 2013 and the other plot was with interspecific hybrid *E. oleifera* × *E. guineensis* (Coari × La Mé) planted in 2014.

### The Behaviour of *Elaeidobius kamerunicus* and Identification of its Natural Enemies

Twenty-four observations were made per day on a biweekly basis to determine the habits of *E. kamerunicus* and to identify its natural enemies. Each observation lasted 10 min. Two inflorescences during anthesis (bloom ≥ 80% of the flowers, phenological stage 607) (Forero *et al.*, 2017; Hormaza *et al.*, 2011) were observed from 06:00 to 18:00 hr. This process took place over a year. Following the observation, the inflorescences in Durawell 3D55 (55 × 16 × 16 cm) pollination bags were isolated (Figure 1a). A total of 32 inflorescences were isolated, cut, and transported to the Cenipalma entomology laboratory located in the CEPV, where the inflorescences' spikelets were removed (Figure 1b-c). All insects by species were separated using a brush,

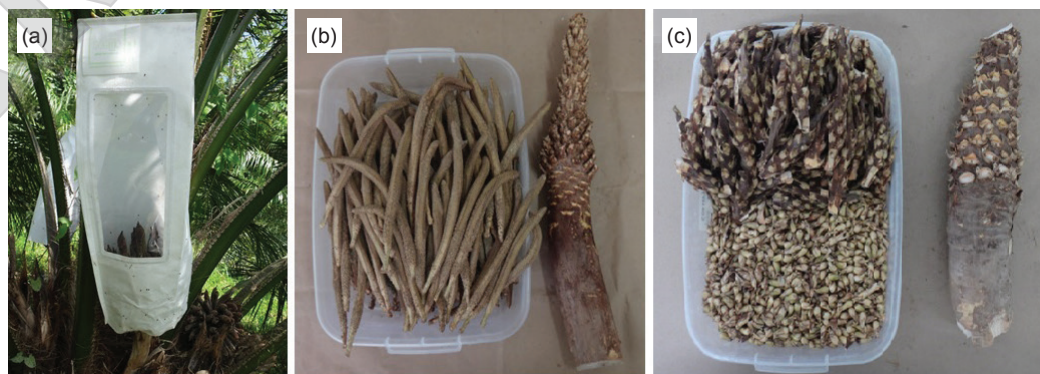


Figure 1. (a) Inflorescence isolation using Durawell 3D55 pollination bags, (b) removal of spikelets from the male inflorescence, and (c) female inflorescence.

counted, and preserved in 70% alcohol. Subsequently, via taxonomic keys, each species was identified (pollinating insects, relatives and natural enemies).

### Biology of *E. kamerunicus*

*Elaeidobius kamerunicus* breeding stock was developed under laboratory conditions ( $26.0 \pm 2.5^\circ\text{C}$ ,  $\text{RH } 69.7 \pm 9.7\%$ ), with adults collected from the field. These adults were divided into 20 individuals (10 males and 10 females) for feeding and oviposition. These were placed in groups into acetate tubes containing a male inflorescence spikelet during the anthesis of the *E. guineensis* cultivar (IRHO 1001) (Figure 2a). The spikelet was set on oasis floral foam to prevent dehydration and was changed every other day.

One hundred newly oviposited eggs were extracted from the male inflorescences and placed individually in Petri dishes containing a damp paper towel. Every hour they were monitored to determine their hatching time and survival rate. The first fifty larvae to emerge were used to establish the developmental time of different larval instars. These were placed individually in Petri dishes containing a flower of the male spikelet during anthesis (as a food source) (Figure 2b). The larva's cephalic capsules were marked using a red fine-tip permanent marker (Sharpie, USA) (Figure 2c). They were checked daily to determine the instar change, which was confirmed by the presence of a new cephalic capsule. Similarly, 100 pupae were placed individually in Petri dishes and monitored until the adults emerged to determine the pupal stage's duration.

The life expectancy of the adults was determined using 100 newly emerged adults. First, their sex was determined, and they were placed in acetate tubes containing a male spikelet at anthesis; these were changed daily to extract the eggs oviposited by the female, and the longevity of the adults was also monitored daily. In addition, any oviposited eggs were extracted from the spikelet using a brush and stereoscope and counted to determine the female oviposition rate.

### Population Fluctuation of *E. kamerunicus*

Every 15 days from each cultivar, samples of *E. kamerunicus* were collected; six inflorescences from the cultivar at anthesis were selected (three male and three female). To estimate the abundance of pollinating insects at the inflorescences, a  $5 \times 5$  cm white interception trap, made with Cartonplast (Compañía de Empaques, Itagui, Antioquia) with a layer of insect adhesive (Super tramp®, Inanalmet®, Bogotá, D.C.), was placed over each inflorescence (Montes *et al.*, 2018) (Figure 3). Every 30 min, the number of *E. kamerunicus* captured per inflorescence was counted. This activity was carried out every day for a year from 06:00 hr to 18:00 hr, taking 24 daily samples per inflorescence. At the CEPV weather station, humidity, temperature and precipitation were recorded.

### Identification of Volatile Organic Compounds

Male and female spikelets at anthesis were collected in plastic bags from both cultivars under investigation between 10:00 hr and 12:00 hr. The spikelets were transported to the CEPV biochemistry laboratory in sterile, 1 L glass bottles to collect volatile organic compounds using headspace solid phase microextraction (HS-SPME), maintaining the bottles at  $40^\circ\text{C}$  temperature for 1 hr. The gas chromatograph (6890N, Agilent Technologies) with a split/splitless injection port and a 5973N mass selective detector identified the volatile organic compounds. It performed injections using a split 10:1 ratio and achieved separation using a  $30 \text{ m} \times 0.25 \text{ mm}$  internal diameter silica capillary column with a  $0.25 \text{ }\mu\text{m}$ -thick film (Shinwa Chemical, Kyoto, Japan). The gas used for transportation was He at a pressure of 30 kPa and stored at  $250^\circ\text{C}$ .

The detector's interface temperature and ionisation voltage was  $250^\circ\text{C}$  and 70 eV, respectively, with electron impact ionisation. The column temperature was increased sequentially with three ramps; it was maintained initially at  $45^\circ\text{C}$  for 5 min. The first increase occurred at a rate of

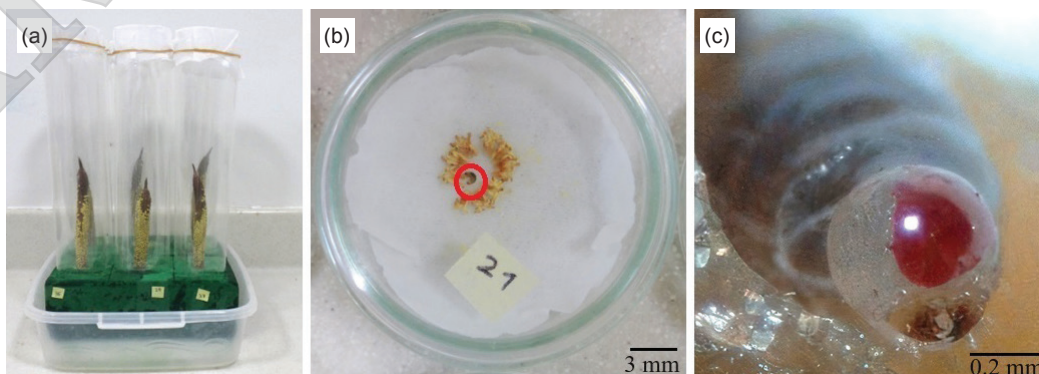


Figure 2. (a) Oviposition unit of *E. kamerunicus* adult breeding stock, (b) Petri dish with flower for the larval stage development, and (c) the cephalic capsule is marked to monitor larval stage instar change.

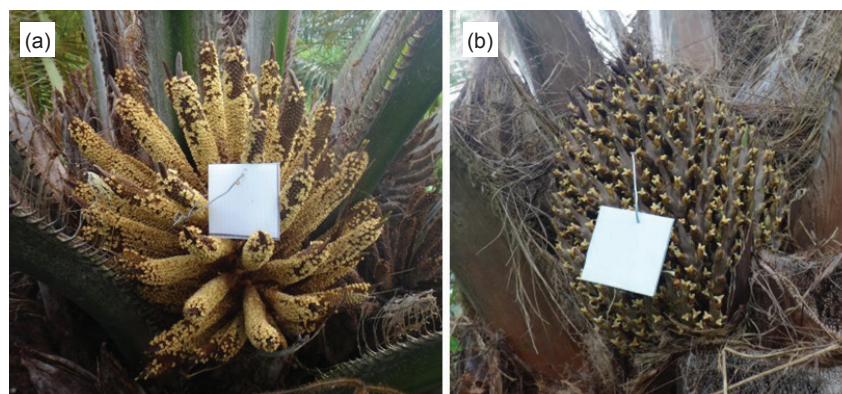


Figure 3. Interception trap placement for *E. guineensis* inflorescences during anthesis: (a) Male inflorescence, and (b) female inflorescence.

4°C/min up to 150°C, with a 2 min hold. A second ramp was at 5°C/min up to 250°C with a 5 min hold. As a final step, the temperature was brought up to 275°C at a rate of 10°C/min with a 20 min hold. The mass spectrometer ran with He as the carrier gas at a 1 mL/min flow rate. Between 40–400 m/z (mass/load ratio) was evaluated the mass range. Once the chromatographic profiles were known, the volatile compounds were detected using an Agilent Technologies 6890N gas chromatograph with a flame ionisation detector following the earlier methodology.

### Statistical Analysis

Data recorded to determine the biology of *E. kamerunicus* were analysed using descriptive statistics. Information on the population of *E. kamerunicus* adults was plotted in population fluctuation graphs for the two cultivars during anthesis. The resulting compounds from volatiles in the cultivars were identified by referencing the Wiley/NIST mass spectrometry database.

## RESULTS AND DISCUSSION

### The Behaviour of *E. kamerunicus* and Identification of its Natural Enemies

Following collection from the male and female inflorescences of the two cultivars, three insect species were identified as pollinators. These were *E. kamerunicus* (Figure 4c), *E. subvittatus* (Figure 4d) and *M. costarricensis* (Figure 4a). *Elaeidobius kamerunicus* was the most prevalent (Table 1) but other insects that visited the inflorescences were also identified. These included *Cyclocephala amazona* Linnaeus, 1767 (Coleoptera: Scarabaeidae) (Figure 5a), *Coproporus* sp. Kraatz, 1857 (Coleoptera: Staphylinidae) (Figure 5b), *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae) (Figure 5c), *Trigona* sp. (Fabricius, 1793) (Hymenoptera: Apidae) (Figure 5d).

Mondragon and Roa (1985) determined that *E. subvittatus*, *M. costarricensis*, *Coproporus* sp., *Orthoperus minutissimus* Matthews, 1899 (Coleoptera: Corylophidae), *Ahasoeris* sp. Des Gozis, (1881) (Coleoptera: Silvanidae), *Thrips* sp. Haliday, 1836 (Thysanoptera), *Smicrips* sp. Le Conte, 1878 (Coleoptera: Smicripidae), *C. amazona*, *Cyclocephala discolor* Herbst, 1792 (Coleoptera: Scarabaeidae); are present in *E. guineensis*. While in the interspecific hybrid, they reported *Orthoperus* sp. Stephens, 1829 (Coleoptera: Corylophidae), as the most abundant. Arústegui *et al.* (2015) determined that *E. kamerunicus*, *E. subvittatus*, *Microporum* sp. Latreille, 1802 (Coleoptera: Nitidulidae), *A. mellifera* and *Melipona* sp. Illiger, 1806 (Hymenoptera: Apidae), are present in the inflorescences of *E. guineensis* (Deli × La Mé) in Peru, and *E. kamerunicus* and *E. subvittatus* are the most abundant pollinators in this cultivar. Egonyu *et al.* (2021) recorded that in Uganda, 23 insect morphospecies present in both sexes of *E. guineensis* inflorescences, including Curculionidae of the genera *Elaeidobius* and *Metamasius* (Coleoptera: Curculionidae), followed by beetles of the family Nitidulidae, Staphylinidae and Scarabidae; dipterans of the family Muscidae, Drosophilidae, and Platystomatidae; and the bee *A. mellifera*.

Natural predators of immature *E. kamerunicus* were observed, such as *Hololepta* sp. Paykull, 1811 (Coleoptera: Histeridae), *Pachycondyla harpax* (Fabricius, 1804) (Hymenoptera: Formicidae), Pseudoscorpiones, Chrysopidae, and Dermaptera, as were predators of adult *E. kamerunicus*, such as spiders (Araneae) (Figure 6). *Pachycondyla harpax* was the most abundant natural enemy observed in the male and female inflorescences of the interspecific hybrid with total counts of  $36.0 \pm 18.4$  and  $7.3 \pm 6.7$  individuals/inflorescence, respectively (Table 1). Muhammad *et al.* (2017) observed and highlighted the rat *Rattus tiomanicus* (Rodentia), the birds *Pycnonotus goiavier* (Pycnonotidae), *Orthotomus ruficeps* (Sylviidae), and *Prinia atrogularis* (Sylviidae), the insects *Cosmolestes picticeps* (Stal, 1859) (Hemiptera: Reduviidae), *Sycanus dichotomus*

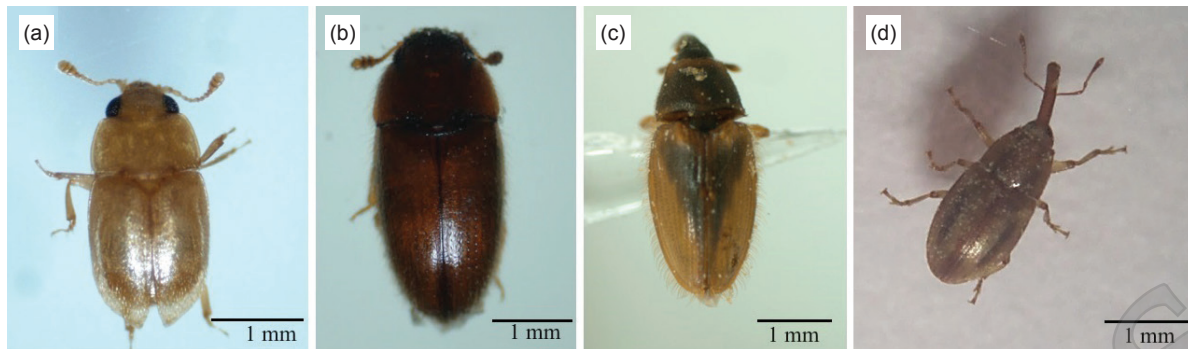


Figure 4. Pollinators collected in oil palm male and female inflorescences during anthesis: (a) *Mystrops costaricensis*, (b) *Mystrops* sp., (c) *E. kamerunicus*, and (d) *E. subvittatus*.

TABLE 1. INSECTS IDENTIFIED IN 32 MALE AND FEMALE *E. guineensis* (IRHO 1001) AND INTERSPECIFIC HYBRID (COARI × LA MÉ) INFLORESCENCES IN THE PALMAR DE LA VIZCAÍNA EXPERIMENTAL FIELD (CENTRAL ZONE)

Cultivar	Type of inflorescence	Type of insect	Insect name	Average number of individuals/ inflorescence day
<i>Elaeis guineensis</i> (IRHO 1001)	Male	Pollinator	<i>Elaeidobius kamerunicus</i> (female)	4325.6 ± 1195.6
			<i>Elaeidobius kamerunicus</i> (male)	1684.0 ± 340.5
			<i>Elaeidobius subvittatus</i>	2.1 ± 2.0
			<i>Mystrops costaricensis</i>	2.0 ± 1.5
	Female	Associated	<i>Apis mellifera</i>	3.5 ± 2.1
			<i>Cyclocephala amazona</i>	77.5 ± 19.1
			<i>Trigona</i> sp.	3.5 ± 3.5
		Pollinator	<i>Elaeidobius kamerunicus</i> (female)	31.8 ± 28.5
			<i>Elaeidobius kamerunicus</i> (male)	11.0 ± 10.8
			Associated	<i>Cyclocephala amazona</i>
	Biological control	Araneae	3.1 ± 3.0	
Interspecific hybrid (Coari × La Mé)	Male	Pollinator	<i>Elaeidobius kamerunicus</i> (female)	728.2 ± 559.4
			<i>Elaeidobius kamerunicus</i> (male)	457.3 ± 399.0
			<i>Elaeidobius subvittatus</i>	2.0 ± 1.0
			<i>Mystrops costaricensis</i>	86.0 ± 73.8
	Female	Associated	Acari	14.5 ± 10.5
			<i>Coproporus</i> sp.	16.0 ± 15.4
			<i>Cyclocephala amazona</i>	2.5 ± 0.7
		Biological control	<i>Trigona</i> sp.	2.0 ± 1.7
			Dermaptera	1.5 ± 0.7
			<i>Pachycondyla harpax</i>	36.0 ± 18.4
Female	Pollinator	<i>Elaeidobius kamerunicus</i> (female)	210.4 ± 147.6	
		<i>Elaeidobius kamerunicus</i> (male)	166.5 ± 92.7	
		<i>Mystrops costaricensis</i>	37.0 ± 9.9	
	Associated	Acari	1.0 ± 0.9	
		<i>Cyclocephala amazona</i>	1.4 ± 1.1	
	Biological control	Araneae	1.8 ± 1.8	
		Dermaptera	1.0 ± 0.8	
		<i>Hololepta</i> sp.	2.1 ± 2.0	
		<i>Pachycondyla harpax</i>	7.3 ± 6.7	

Amyot and Serville, 1843 (Hemiptera: Reduviidae), *Pheidole megacephala* Fabricius, 1793 (Hymenoptera: Formicidae) and the arachnids *Argiope* sp.

(Araneidae) and *Leucauge grata* (Tetragnathidae); as the predators of *E. kamerunicus* present in the palm agroecosystem in Malaysia.

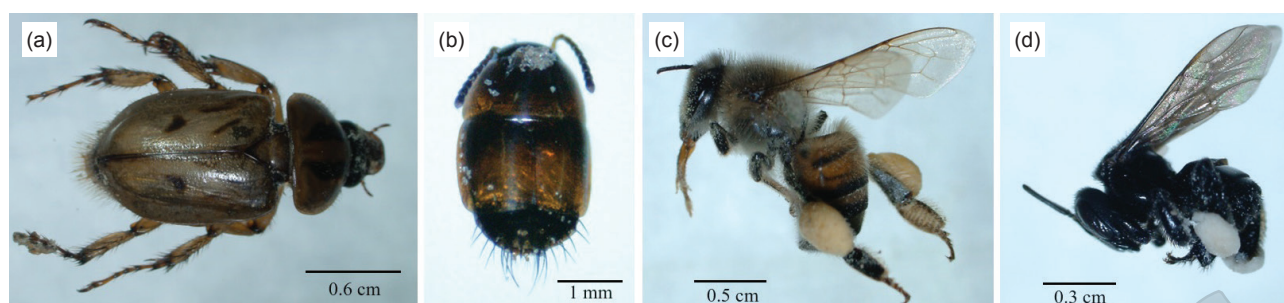


Figure 5. Insects associated with *Elaeis guineensis* and interspecific hybrid male and female inflorescences: (a) *Cyclocephala amazona*, (b) *Coproporus sp.*, (c) *Apis mellifera*, and (d) *Trigona sp.*

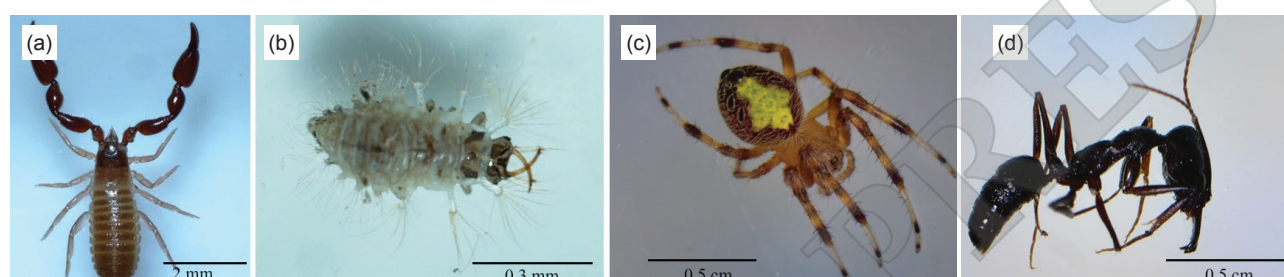


Figure 6. Natural enemies of pollinators: (a) *Pseudoscorpionida*, (b) *Chrysopidae*, (c) *Araneae*, and (d) *Pachycondyla harpax*.

Observations suggested that the activity of *E. kamerunicus* was related to environmental conditions. For instance, on days when the sky had thick clouds, the number of *E. kamerunicus* in the inflorescences was reduced. We did not observe the arrival of insects to the inflorescences on rainy days. On sunny days, their flight was more active. The insects' visits to the inflorescences were recorded from 08:00 hr to 12:00 hr, and the visits to inflorescences during anthesis took place intermittently in groups for the two cultivars studied. When the volatile compounds emitted by inflorescences were strong, pollinators arrived and settled on the peduncle of the inflorescence or the petiolar leaf fibres (Figure 7b); however, when these vapours were weak, pollinators settled on the underside of the leaves closest to the inflorescence (Figure 7a). Subsequently, they walked to the inflorescence at anthesis and hide at the base of the spikelet, where the first male flowers have bloomed, to feed on pollen. Pollinating insects reached inflorescences covered in pollen, indicating that they have come from other male inflorescences at anthesis.

Between 09:30 hr and 11:30 hr was estimated to be the time that *E. kamerunicus* males roam the flowers in search of females for copulation (Figure 7d). Later, the females oviposit their eggs on the stamens of the flowers at the base of the spikelet, where they use their proboscis to scrape at the petals and sepals to cover the oviposited eggs (Figures 7e-f). Abd *et al.* (2022) determined that the more active

*E. kamerunicus* females spent a lot of time feeding and moving around to find the oviposition site, while the males groomed their legs to improve their grasping capacity during the copulation process, as well as to improve their sensory capacities (chemo and mechanoreceptors), they were more active after feeding and resting from the mating process.

After 12:00 hr, *E. kamerunicus* activity decreased considerably. At night (18:00 hr and 20:00 hr), insect movement was observed over the entire inflorescence, although the arrival of new pollinators was not evident. We found that environmental conditions play an essential role in regulating the activity of *E. kamerunicus*. Prada *et al.* (1998) reported observing similar behaviour in *E. kamerunicus* adults which visited inflorescences during the day, especially during hours of high brightness. However, they did not observe any activity on rainy or cloudy days.

The behaviour of the pollinating insects in the female inflorescence of the interspecific hybrid was minimal since few pollinators visited it. The volatiles like those emitted by the male inflorescences attracted these pollinators. They traversed the inflorescence, leaving pollen all over the flower, and finally, they searched for other flowers.

### Biology of *E. kamerunicus*

The eggs of *E. kamerunicus* are a light cream color (Figure 8), measuring  $0.6 \pm 0.02 \times 0.4 \pm 0.01$  mm (Table 2). They develop in  $0.5 \pm 0.2$  days (average  $\pm$  standard deviation) and have a hatching rate of

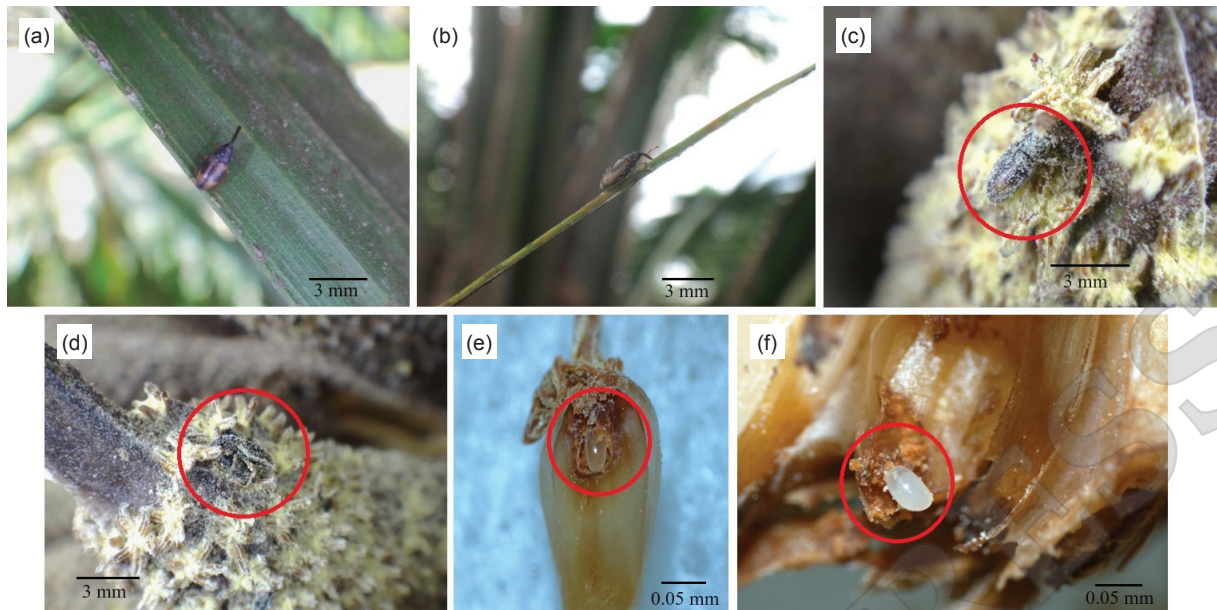


Figure 7. *Elaeidobius kamerunicus* habits: (a) Adult perched on a palm leaflet, (b) adult perched on leaf petiole fibers, (c) adult feeding on pollen, (d) copulation, (e) egg oviposited inside the male flower, and (f) newly oviposited egg.

TABLE 2. MEASUREMENTS OF *E. kamerunicus* AT VARIOUS DEVELOPMENT STAGES GROWN ON MALE INFLORESCENCES OF *E. guineensis* IRHO 1001 (26.0 ± 2.5°C, RH 69.7 ± 9.7%)

Stage of development	Body (mm)		Cephalic capsule (mm)	
	Length	Width	Length	Width
Egg	0.6 ± 0.02	0.4 ± 0.01		
Larva (first-instar)	1.2 ± 0.07		0.3 ± 0.01	0.3 ± 0.01
Larva (second-instar)	2.2 ± 0.70		0.4 ± 0.01	0.4 ± 0.01
Larva (third-instar)	3.9 ± 0.20		0.5 ± 0.01	0.6 ± 0.01
Pupa	2.9 ± 0.10	1.3 ± 0.03		

93.4%. In contrast, Tou *et al.* (2011) determined that the eggs developed in 0.2 days (5.9 hr) and presented an average hatching rate of 97.8%; this high percentage of hatching is due to the protection with sawdust that the female performs when ovipositing, generating better conditions for the development of the egg.

The larval stage shows three instars (Figure 8); they feed on the stamens and the internal parts of the male flower in anthesis. The first-instar larva is translucent and is 1.2 ± 0.07 mm long (Table 2). It has a development time of 0.5 ± 0.1 days and a survival rate of 77.0%. The second-instar larva is amber in color and is 2.2 ± 0.7 mm long (Table 2). It develops in 0.9 ± 0.1 days and has a survival rate of 87.0%. Finally, the third instar is dark amber, approximately 3.9 ± 0.2 mm long (Table 2). It takes 4.6 ± 0.5 days to develop and has a 63.2% survival rate. For this stage of development, Tuo *et al.* (2011) reported three larval stages with a time of 0.8 days (18.2 hr) for the first instar, 1.2 days for the second instar, 1.1 days for the third instar and 4.7 days in the prepupal state.

The pupae develop within the male flower. They are exarate and yellow when they first pupate. Over

time, we observed the development of the eyes and elytra. The pupae turn a grayish brown and later acquire their adult appearance (Figure 8). The pupal stage takes 2.1 ± 0.3 days to develop (Figure 8), with a survival rate of 94.0%. Tuo *et al.* (2011) reported times similar to those found in this study for this stage of development (2.03 days). The life cycle from egg to adult was 8.6 days. The results were different from those reported by Tuo *et al.* (2011), who reported a total life cycle time of 10.3 ± 0.3 days (27.4 ± 0.7°C, RH 75.2 ± 2.5%); this difference of 1.7 days probably is due to the different environmental conditions in which we carried out the studies.

It was observed that *E. kamerunicus* males have two calluses in the proximal region of the elytra, golden-colored marginal setae and a tuft-shaped crest in the dorsal part of the elytra. In contrast, these features are absent in females. Otherwise, males and females are similar in appearance; they have blackheads and brown elytra with two large black maculations (Figure 8). Adult life expectancy was 40.8 ± 23.0 and 51.4 ± 18.7 days for males and females, respectively (Figure 8), while Tuo *et al.*

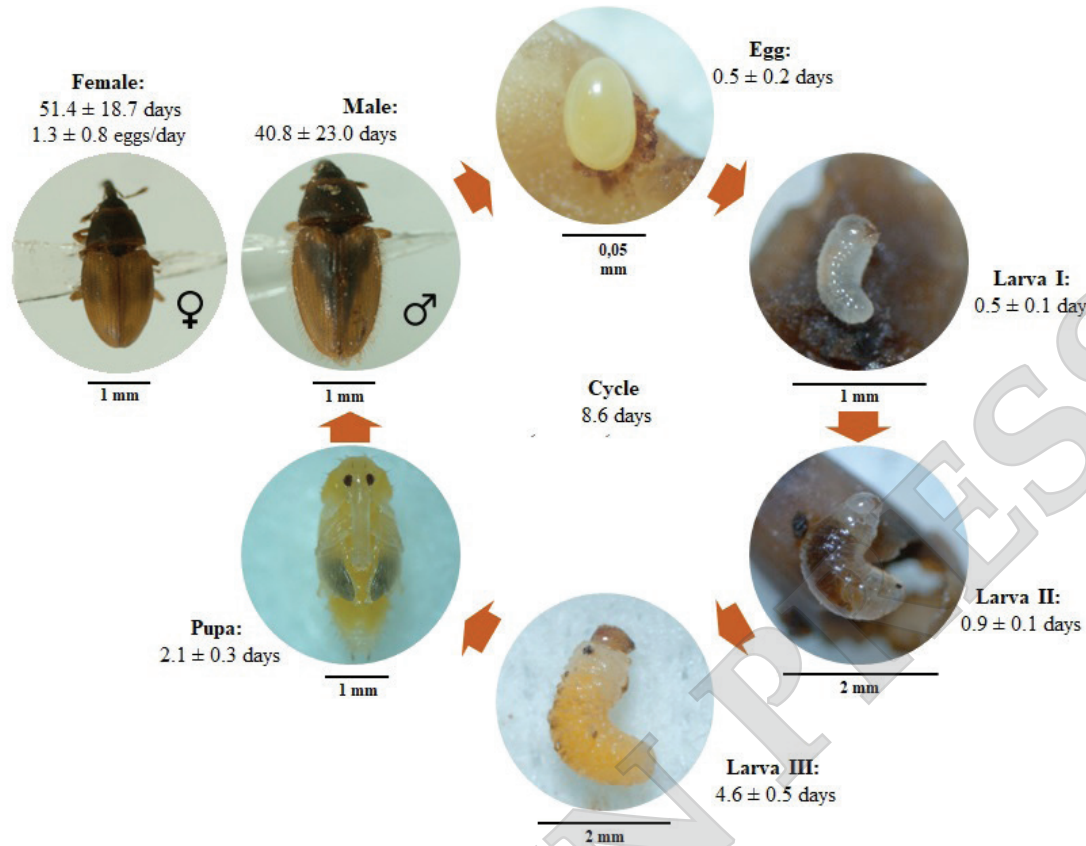


Figure 8. Life cycle of *E. kamerunicus* grown on male inflorescences of *E. guineensis* (IRHO 1001) (26.0 ± 2.5°C, RH 69.7 ± 9.7%).

(2011) reported longevity of 28.0 days for males and 31.2 days for females. We determined that *E. kamerunicus* females had an oviposition rate of 1.3 ± 0.8 eggs/day; these results coincide with what was reported by Tuo *et al.* (2011), who determined an average oviposition rate of 57.6 eggs with 1.8 daily eggs oviposited per female. Hussein *et al.* (1991) reported an average of 35.0 eggs per female of *E. kamerunicus* during her longevity. Females begin their oviposition after the fourth day of their emergence. The male-to-female sex ratio was 1:2.

The total mortality of the *E. kamerunicus* generation studied was 62.8%, with the third instar showing the highest mortality percentage. These results coincide with those reported by Husein *et al.* (1991), who obtained 60% mortality in the larvae during the breeding of *E. kamerunicus*, with the highest mortality rate in the first larval stage. This high mortality in the larval stage is probably due to the manipulation that monitors the change in instar and food.

#### Population Fluctuation of *E. kamerunicus*

It was determined that *E. kamerunicus* adults visit the inflorescences of the *E. guineensis* (IRHO 1001) cultivar in higher numbers during anthesis compared to the interspecific hybrid (Coari ×

La Mé). These results are similar to those of Mondragón and Roa (1985) and Yue *et al.* (2015), who determined that the number of insects attracted by *E. guineensis* is higher and more variable, compared to the interspecific hybrid, which attracts fewer insects and less variety. Also, Prasetyo *et al.* (2019) found that *E. guineensis* inflorescences were attractive to *E. kamerunicus*, while the presence of *E. kamerunicus* was lower in the *E. guineensis* × *E. oleifera* backcrosses. These population differences in *E. kamerunicus* between the evaluated cultivars are due to the presence, absence, and concentration of organic volatiles emitted by the inflorescences of each cultivar. In addition, it is essential to know the role played by these organic compounds emitted by the inflorescences in the interaction with the adults of *E. kamerunicus*, Ullah *et al.* (2015) have reported that the benzaldehyde compound is an insect repellent, and this is present in the interspecific hybrid, this may explain why the lowest visit rate of pollinating insects occurred in this material.

The male inflorescences of the *E. guineensis* cultivar were visited by 6010 *E. kamerunicus* adults during one day of anthesis, whereas approximately 42.8 of *E. kamerunicus* individuals frequent the female inflorescence. In contrast, Prada *et al.* (1998) determined that about 31 321 individuals of *E. kamerunicus* visit the female inflorescence of *E. guineensis* on one day of anthesis. Sánchez



*et al.* (2004) determined that the populations of *E. kamerunicus* per male inflorescence in anthesis ranged from 9606 to 156 753 individuals. Also, Arústegui *et al.* (2015) reported 124 527 and 224 219 adults of *E. kamerunicus* in male and female inflorescences in the anthesis of *E. guineensis* (Deli × La Mé), respectively.

Various factors, including the cultivated material, physiology of the inflorescence, number of male inflorescences at anthesis per hectare, climate (temperature, rainfall and dry periods), crop management practices (use of pesticides), the life cycle of pollinators, volatiles emitted by the inflorescences and the presence of natural enemies, influence the population fluctuation of *E. kamerunicus* (Nurul *et al.*, 2019; Prasetyo *et al.*, 2019; Sánchez *et al.*, 2004). The ratio of females to males is 2.9:1.0 for *E. guineensis*, whereas the ratio is 1.6:1.0 for the interspecific hybrid.

It was observed that there is a better activity of pollinating insects on the male inflorescence of *E. guineensis* between 09:30 hr and 12:00 hr (Figures 9a-b). The number of *E. kamerunicus* adults visiting the *E. guineensis* and the interspecific hybrid male inflorescences peaked at 11:00 hr (Figure 9). Prada *et al.* (1998), Sánchez *et al.* (2004), and Yue *et al.* (2015) determined that the time of maximum activity of *E. kamerunicus* was between 10:30 hr and 12:00 hr. Notably, the population dynamics of pollinating insects are regulated by the volatile compounds emitted by inflorescences. Therefore, the higher the emission of volatile compounds, the greater the activity of pollinators.

### Identification of Volatile Organic Compounds

The chromatographic elution order of the main compounds identified is as follows: Ethyl salicylate, estragole, *cis*-Anethole, and *trans*-Anethole. In our results, estragole corresponds to the highest peak area, revealing it to be the most prevalent among the volatile organic compounds detected. Based on the chromatographic peak areas, the volatile organic compound emitted by the inflorescence differs depending on whether it is male or female. Female inflorescences emit fewer compounds than male inflorescences (Figure 10). The variation in pollinators visiting each inflorescence correlates with this difference (Figure 10).

The HS-SPME test facilitated a qualitative characterisation of the compounds emitted by inflorescences, and their subsequent identification was obtained using GC-MS. This tool has proven helpful in identifying volatile organic compounds involved in the interaction between insects and inflorescences (Basaglia *et al.*, 2014). Studies conducted by Lajis *et al.* (1985) and Yue *et al.* (2015) suggested that estragole was the compound

responsible for the *E. kamerunicus* insect's attraction. In our results, estragole corresponds to the highest peak area, revealing it to be the most prevalent among the volatile compounds detected.

Furthermore, it was observed that the interspecific hybrid (Coari × La Mé) inflorescences emit minority compounds not detected in the *E. guineensis* (IRHO 1001) inflorescences. These were identified as styrene, benzaldehyde, isopropyl methoxy pyrazine, dimethyl nonatriene, isobutyl methoxy pyrazine, beta-elemene and triacetin. Methoxy pyrazines have been reported as attractants to some pollinating insects (Maia *et al.*, 2018). In contrast, benzaldehyde has been reported as a repellent and insecticide against *Galleria mellonella* (Lepidoptera: Pyralidae), causing 100% insect mortality was observed at 108 hours after the injections (Ullah *et al.*, 2015). The interactions of these compounds with *E. kamerunicus* have not been reported to date. Therefore, whether these compounds have an attractive or repulsive effect on the insect remains unknown. These differences in the presence and concentration of volatile organic compounds could explain the population dynamics of *E. kamerunicus* in each cultivar.

### CONCLUSION

*Elaeidobius kamerunicus* adults visit the inflorescences of the cultivar *E. guineensis* (IRHO 1001) during anthesis in more significant numbers than the interspecific hybrid (Coari × La Mé). The ratio of females to males was 2.9:1.0 for *E. guineensis* and 1.6:1.0 for the interspecific hybrid.

The most significant activity of *E. kamerunicus* was between 09:30 hr and 12:00 hr. They copulate between 09:30 hr and 11:30 hr, and the females oviposit on the stamens of the male flowers. The life cycle of *Elaeidobius kamerunicus* from egg to pupa was 8.6 days, with a life expectancy of 40.8 days for males and 51.4 days for females. The oviposition rate of the females was 1.3 eggs/day, with a development time of 0.5 days.

*Pachycondyla harpax* was the most abundant natural enemy observed in the inflorescences, and the other identified enemies were *Hololepta* sp., Chrysopidae, Dermaptera, Araneae and Pseudoscorpiones. Methyl salicylate, estragole, *cis*-anethole, and *trans*-anethole were identified as the main compounds emitted by the male and female inflorescences of the two cultivars studied.

The behaviour of the adult population of *E. kamerunicus* in the two cultivars studied could be modulated by the emission of organic compounds identified from the male and female inflorescences. The knowledge of these compounds is essential for developing attractants that improve interspecific hybrids' productivity.

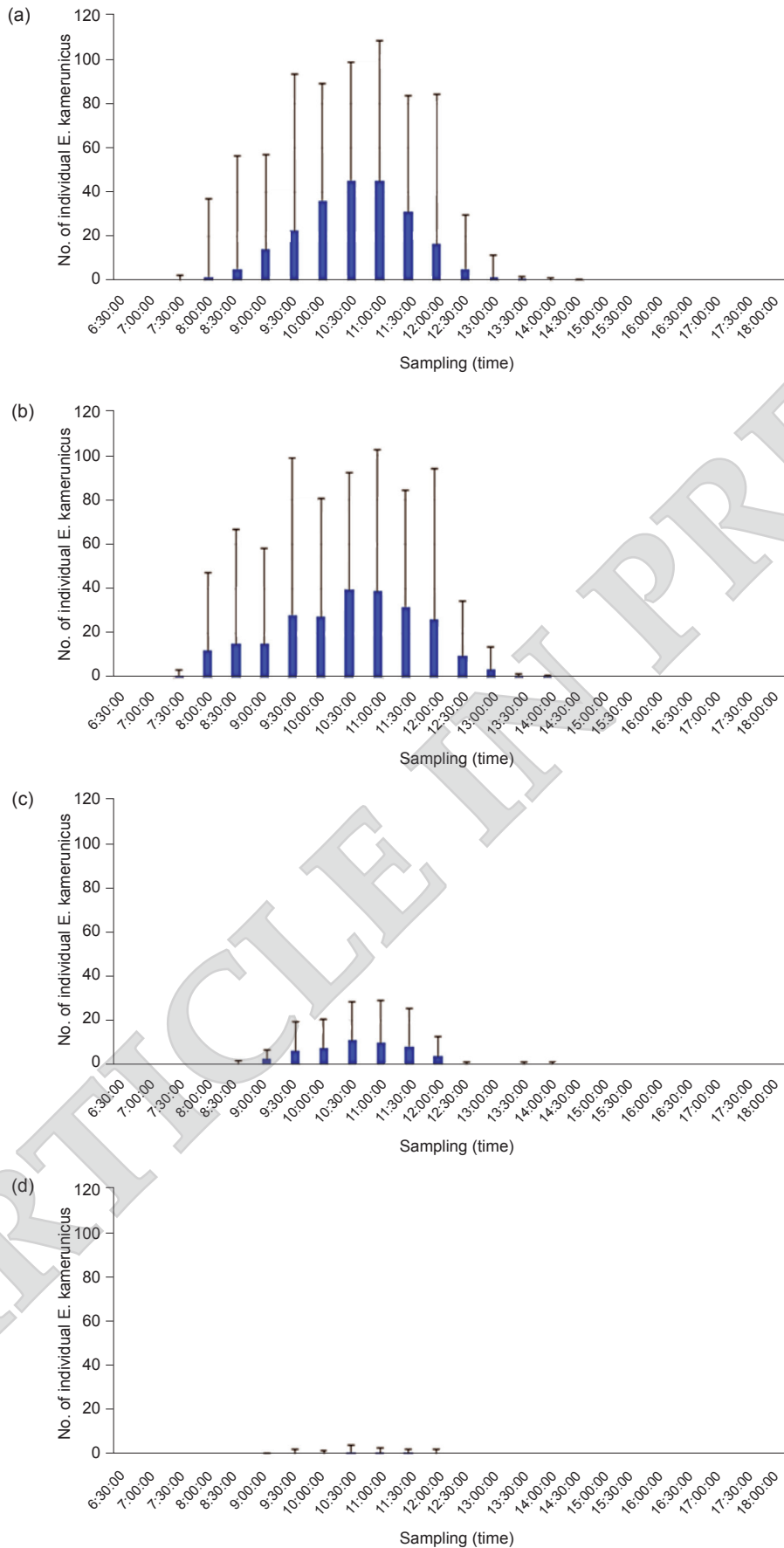


Figure 9. Population fluctuation of *Elaeidobius kamerunicus* on inflorescences of *E. guineensis* (IRHO 1001) during anthesis; (a) male inflorescence, (b) female inflorescence, and in inflorescences of the interspecific hybrid (Coari × La Mé); (c) male inflorescence, and (d) female inflorescence.

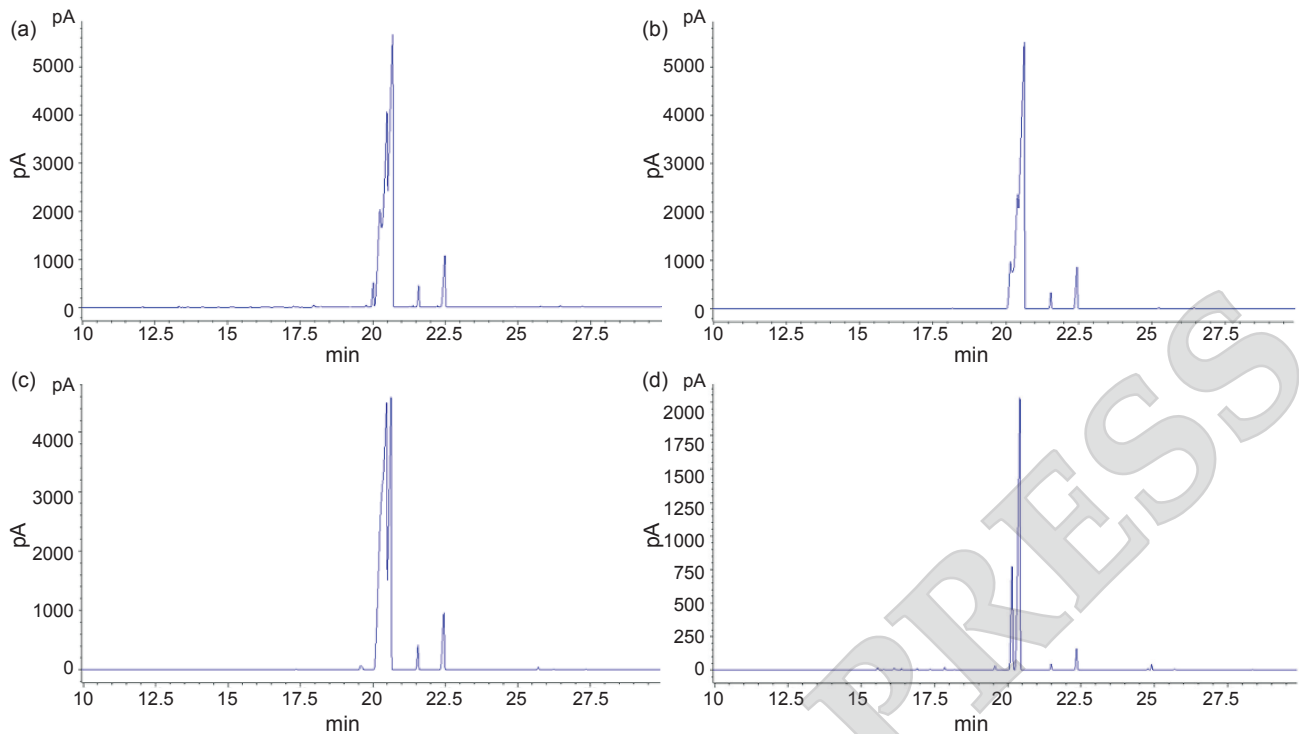


Figure 10. Chromatogram of the inflorescences of *Elaeis guineensis* (IRHO 1001) during anthesis: (a) Male inflorescence and (b) female inflorescence. From the interspecific hybrid (Coari  $\times$  La Mé): (c) Male inflorescence and (d) female inflorescence.

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CAS-C, NFU-M, and JDC-M carried out the experiment. CAS-S and NFU-M wrote the manuscript with support from AEB-P and AM-R. All authors provided critical feedback and helped shape the research, analysis and manuscript. AEB-P and AM-R supervised the project.

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